IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Harris *et al.* Confirmation No. 4949

Serial No.: 09/615,571 Group Art Unit: 1632

Filed: July 13, 2000 Examiner: S.D. Priebe

For: Polypeptides Having Phospholipase B Activity And Nucleic Acids Encoding Same

AMENDMENT

Commissioner for Patents
P.O. Box 1450
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Sir:

This communication is in response to the Advisory Action dated July 12, 2004. Claims 100, 102-107, 109-112, 114-117, and 119-128 are pending in the present application.

It is respectfully submitted that the present amendment presents no new issues or new matter and places this case in condition for allowance. Reconsideration of the application in view of the following remarks is requested.

I. The Rejection of Claims 100, 102-104, 109-111, 114-115, and 120-124 under 35 U.S.C. § 112, First Paragraph

Claims 100, 102-104, 109-111, 114-115, and 120-124 stand rejected under 35 U.S.C. § 112, first paragraph, "as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention" for the reasons of record. The Office states:

> Applicant states that three structural features have been used in the art to define relatedness of genes and proteins, % identity between amino acid or nucleic acid sequences and hybridization under stringent conditions. However, these criteria apply to naturally occurring polypeptides and nucleic acids where the sequences are known to be wild-type (or functional), while the claims also embrace man-made variants of SEQ ID NO: 1, which is the sole naturally occurring nucleic acid sequence disclosed. No artificial amino acid sequences are disclosed. Furthermore, as Applicant states, the prior art was devoid of a phopholipase B or nucleotide sequence encoding such that

> shared any significant homology SEQ ID NO: 2 or SEQ ID NO: 1, respectively. The specification also fails to disclose any amino acid sequence differing from SEQ ID NO: 2 or nucleic acid encoding such a differing amino acid sequence, whether a natural or man-made sequence. Consequently, there is nothing to which one can compare SEQ ID NO: 2, whether by sequence homology or by hybridization. There is no information or evidence from either the prior art or the specification that would allow one of skill in the art to predict with any reliability whether an amino acid sequence differing from SEQ ID NO: 2 in up to 10% of its amino acids would have phospholipase B activity.

This rejection is respectfully traversed for reasons of record and for additional reasons discussed below.

The present invention relates to isolated nucleic acid sequences encoding a polypeptide having phospholipase B activity, selected from the group consisting of:

(a) a nucleic acid sequence encoding a polypeptide having an amino acid sequence which has at least 90% identity with amino acids 20 to 464 of SEQ ID NO:2;

(b) a nucleic acid sequence having at least 90% homology with nucleotides 568 to 2045 of SEQ ID NO:1; and

(c) a nucleic acid sequence which hybridizes under at least medium-high stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO:1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO:1, or (iii) a complementary strand of (i) or (ii).

Applicants submit that the specification complies with the written description requirement. The Federal Circuit provides that the written description requirement for a genus of DNAs is met by a recitation of a representative number of DNAs, defined by nucleotide sequence, falling within the scope of the genus or by a recitation of structural features common to the members of the genus. See, e.g., *University of California v. Eli Lilly and Co.*, 43 U.S.P.Q.2d 1398, 1404 (Fed. Cir. 1997); *Enzo Biochem v. Gen-Probe Inc.*, 63 U.S.P.Q.2d 1609, 1613 (Fed. Cir. 2002).

As Applicant has stated in the record, the three structural features of (1) percent identity of the amino acid sequences encoded by the genes, (2) percent homology of the nucleic acid sequences of the genes, and (3) nucleic acid hybridizations under defined stringent conditions have been used for decades by the scientific community to define the relatedness of genes and their products and to identify complementary strands of genes encoding the same or similar enzyme or protein function. These structural features have been used to predict the function of polypeptides encoded by novel genes, and to place them in an existing genus.

In the claims at issue, Applicants provide a recitation of three structural features

common to the claimed genus: (1) a nucleic acid sequence encoding a polypeptide having an amino acid sequence which has at least 90% identity with amino acids 20 to 464 of SEQ ID NO:2; (2) a nucleic acid sequence having at least 90% homology with nucleotides 568 to 2045 of SEQ ID NO:1; and (3) a nucleic acid sequence which hybridizes under medium-high stringency conditions with (i) nucleotides 568 to 2045 of SEQ ID NO:1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO:1, or (iii) a complementary strand of (i) or (ii).

The Office argues that the specification does not adequately describe those structural, physical, and chemical characteristics of the claimed nucleic acids or the enzyme they encode to distinguish them from nucleic acid sequences which are not claimed because there is no information or evidence from either the prior art or the specification that would allow one of skill in the art to predict with any reliability whether an amino acid sequence differing from SEQ ID NO: 2 in up to 10% of its amino acids would have phospholipase B activity.

In support of Applicant's arguments that the claimed structural features provide a correlation between function and structure and predict with reliability that an amino acid sequence differing from SEQ ID NO: 2 in up to 10% of its amino acids would have phospholipase B activity is the Declaration of Dr. Randy Berka of November 19, 2004.

Dr. Berka states that "the recited structural relationships of (1) percent identity of the amino acid sequences encoded by the genes, (2) percent homology of the nucleic acid sequences of the genes, and (3) nucleic acid hybridizations under defined stringent conditions to identify complementary strands of genes encoding the same or similar enzyme or protein function are far from arbitrary, and they are based on very conservative, logical and rational scientific deductions that are supported by detailed statistical analyses reported in the scientific literature. ... Since the collective structural properties of proteins (circumscribed by their primary structure) are responsible for their biological activities, proteins that share a high degree of amino acid sequence identity are known with reasonable certainty to possess the same biochemical/biological activities." Dr. Berka provides several observations to support this statement.

First, there are literally hundreds of reports in which investigators have used nucleic acids probes from one species to clone genes encoding a homologous protein/enzyme from a heterologous source.

Second, homologues of a newly sequenced gene product can be identified *via* database searches using BLAST, Smith-Waterman and other computer algorithms and structure and function assigned to the gene product based on the concept that sequence similarity implies structural and functional similarity.

Third, functional classification is conserved over a range of sequence similarity and biological/biochemical function diverges only when sequence similarities are low enough that they have no statistical significance. Dr. Berka provides seven examples in which BLASTP was used to query a publicly available sequence database (Protein Information Resource, PIR-NREF, GeneseqP) with different fungal enzymes (amylase, acid protease, glucoamylase, exocellobiohydrolase, endoglucanase, phytase, and lipase) and asked if homologous proteins with at least 90% identity possessed the same biological/biochemical activity. The results summarized in Appendix 1 of Dr. Berka's Declaration show clearly and convincingly that proteins with 90% sequence identity are annotated to have the same activity.

Dr. Berka provides further evidence by Wilson *et al.*, 2000, *J. Mol. Biol.* 297: 233-249, which established a clear relationship between sequence similarity and functional similarity. Wilson *et al.* found that functional identity is conserved down to approximately 40% amino acid sequence identity, and that among proteins that share 50-100% sequence identity, function is conserved in almost all, which extended the previous observations of Chothia and Lesk, 1986, *EMBO J.* 5: 823-826, which compared 32 pairs of homologous proteins and found that with pairs whose sequence identity is greater than 50%, at least 90% of the residues lie in structurally common cores.

One essential feature of the claimed invention is isolated nucleic acids that hybridize to nucleotides 568 to 2045 of SEQ ID NO: 1 under the specified stringency conditions and have a specified percent homology and encode polypeptides with a specific function, *i.e.*, phospholipase B activity. Dr. Berka provides that the claim is drawn to nucleic acids all of which must hybridize with nucleotides 568 to 2045 of SEQ ID NO: 1 and must encode a protein with phospholipase B activity. Dr. Berka states: "The logic of the patent examiner's argument that claimed synthetic sequences of 80-90% sequence identity are unusable as probes is flawed, since the claimed proteins derived from the resulting genes must have phoshpholipase B activity. The fact that two sequences that share 80% identity to SEQ ID NO: 1 may have as little as 40% sequence identity to each other is irrelevant as long as the gene they detect shares 80-90% identity with SEQ ID NO: 1 and the corresponding gene product exhibits phospholipase activity."

Dr. Berka also provides that one of ordinary skill in the art would not expect any substantial variation among species encompassed within the scope of the claims because the specified hybridization and percent identity conditions set forth in the claims yield structurally similar DNAs and proteins. He supports this statement with several citations where genes coding for proteins of similar function were cloned on the basis of hybridization to heterologous probes under a variety of stringency conditions to demonstrate that cross-

hybridization is to a significant extent predictive of gene relatedness, and gene relatedness is in turn predictive of functional similarity. As the publications of Chothia and Lesk, 1986, *EMBO J.* 5: 823-826; Bork *et al.*, 1994, *Curr. Opin. Struct. Biol.* 4: 393-403; Bork *et al.*, 1998, *J. Mol. Biol.* 283: 707-725, and Wilson *et al.*, 2000, *J. Mol. Biol.* 297: 233-249 show, it is virtually certain that proteins with a high degree of amino acid sequence identity (>50%) have the same biological/biochemical function.

The Office argues that while one of skill in the art can readily envision numerable species of nucleic acid sequences that are at least 90% identical to the recited reference nucleotide sequence and that encode a polypeptide that is at least 90% identical to the recited reference amino acid sequence, one cannot envision which of these also encode a polypeptide with phospholipase B activity. Dr. Berka disagrees with this argument and states: "[T]he work of Wilson *et al.*, 2000, *J. Mol. Biol.* 297: 233-249, demonstrates convincingly that proteins which share 50-100% sequence identity almost always represent molecules with the same biochemical/biological function."

The Office argues that since there are no other examples of a phospholipase B known that have structural homology with SEQ ID NO: 2, it is not possible to even guess at the amino acid residues which are critical to its structure or function based on sequence conservation. Dr. Berka states: "Guo *et al.*, 2004, *Proc. Nat. Acad Sci USA* 101: 9205-9210, observed that various residues of a protein are differentially sensitive to substitutions, and that tolerance of the entire protein to random change can be characterized by a probabilistic relationship termed the "*x*-factor." The *x*-factor is broadly defined as the probability that a random amino acid replacement will lead to functional inactivation. Moreover, they determined the *x*-factor to be 34% $\pm$ 6%. Contrary to the Office's contention that random (even conservative) changes in a protein in the absence of structural information would adversely affect folding and/or activity, the findings of Guo *et al.* (2004) support the contrary, *i.e.*, that proteins are generally tolerant to random amino acid substitutions, and the probability of destroying protein function is surprisingly small."

The Office states: "The specification also fails to disclose any amino acid sequence differing from SEQ ID NO: 2 or nucleic acid encoding such a differing amino acid sequence, whether a natural or man-made sequence. Consequently, there is nothing to which one can compare SEQ ID NO: 2, whether by sequence homology or by hybridization." Dr. Berka provides that phospholipase B enzyme described in the present application is novel and represents the first member of a new "genus", but the enzyme harbors sequence and structural motifs that are well known for enzymes of the phosphoesterase class (which contains not only phospholipase B, but also some phospholipase C, and other

phosphomonoesterase enzymes). Using a standard software tool (HMMPFAM) that is well known in the art (Sonhammer *et al.*, 1998, *Nucleic Acids Research* 26: 320-322), Dr. Berka generated a profile of conserved amino acid motifs representing highly conserved sequences in this family. As noted by Guo *et al.* (2004), such highly conserved segments may be critical for enzyme activity or biological function, and they are expected to be less tolerant for substitutions (see Appendix 2 for an example of this analysis). In fact, the findings of Guo *et al.* (2004), as described in Dr. Berka's Declaration, support the contention that proteins are generally tolerant to amino acid substitutions, and the probability of destroying protein function is surprisingly small.

Dr Berka states: "A skilled person with such information could easily prepare a variant of SEQ ID NO: 2 containing a deletion, insertion, and/or substitution of one or more amino acid residues. The specification on page 7, lines 8-30, provides that conservative amino acid substitutions can be made that do not significantly affect the folding and/or activity of SEQ ID NO: 2 and provide examples of conservative substitutions within the group of basic amino acids, acidic amino acids, polar amino acids, hydrophobic amino acids, aromatic amino acids, and small amino acids. The specification on page 13, line 9, to page 14, line 4, also describes methods for identifying amino acid residues essential to the activity of a phospholipase B." Dr. Berka further states: "The Office is, therefore, incorrect in stating that it is not possible to even guess at the amino acid residues which are critical to its structure or function based on sequence conservation."

In the claims at issue, each of the claimed structural features (percent identity, percent homology, and hybridization) specifies, therefore, a genus of structurally- and functionally-related enzymes having phospholipase B activity. Since the claimed structural features provide a correlation between function and structure, the written description requirement is satisfied.

Limiting the literal scope of protection of such a new genus to the nucleic acid sequence of SEQ ID NO: 1 or the amino acid sequence of SEQ ID NO: 2 provides little incentive to an Applicant to seek patent protection because biological diversity dictates that there will be natural variation in the sequences of other homologous genes existing in nature that are structurally- and functionally-related.

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

II. The Rejection of Claims 100, 102-104, 109-111, 114-115, 117 and 119-124

under 35 U.S.C. § 112, First Paragraph

Claims 100, 102-104, 109-111, 114-115, 117 and 119-124 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for a nucleic acid sequence encoding phospholipase B wherein either the nucleic acid sequence comprises nucleotides 568 to 2045 of SEQ ID NO: 1 or the polypeptide comprises amino acids 20-464 of SEQ ID NO: 2, does not reasonably provide enablement for any other embodiments lying outside this scope for the reasons of record. The Office states:

> Applicant asserts that at the time the invention was made (Oct. 1999) that it was routine for one of skill in the art to make multiple modifications to an amino acid sequence, citing a reference published in 2003. Applicant is reminded that the enablement requirement must have been met in Oct. of 1999. Applicant cites Example 2 as showing how to make a claimed nucleic acid. However, this example shows obtaining complete genomic clones from the same source organism as a partial clone - one that encodes SEQ ID NO: 2. It does not demonstrate using a nucleic acid of SEQ ID NO: 1 to isolate a claimed nucleic acid from a different source, nor does the specification identify a source, from which one would be able to isolate a claimed nucleic acid, other than A. oryzae. More importantly, the claims are not limited to sequences obtainable from a natural source, and the example does not teach how to make a nucleic acid readable on the claims that cannot be found in nature and encodes a different amino acid sequence than SEQ ID NO: 2. Such unknown artificial sequences would have been expected to make up the vast majority of the species readable on the claims.

This rejection is respectfully traversed for reasons of record and for additional reasons discussed below.

"[A] specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of section 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support." *In re Marzocchi*, 169 USPQ 367, 369 (CCPA 1971).

"The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art ... The test is not quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed to enable the determination of how to practice a desired embodiment of the invention claimed ..." *Ex parte Jackson*, 217 U.S.P.Q. 804 (Bd. Pat. App. 1982).

The reasoning provided in the Office is that the specification "does not demonstrate using a nucleic acid of SEQ ID NO: 1 to isolate a claimed nucleic acid from a different source, nor does the specification identify a source, from which one would be able to isolate a claimed nucleic acid, other than *A. oryzae*" and "the claims are not limited to sequences obtainable from a natural source, and the example does not teach how to make a nucleic acid readable on the claims that cannot be found in nature and encodes a different amino acid sequence than SEQ ID NO: 2." Applicants respectfully submit that this reasoning is not sufficient to render the claims nonenabled.

The specification contains an extensive disclosure of techniques which are well known in the art and indeed routine for persons of ordinary skill in the art for identifying other nucleotides of the present invention. Applicants describe methods for preparing and probing DNA libraries (Example 1-2); for isolating nucleic acids encoding the phospholipases (Example 3); for determining cross-hybridization of the nucleic acids encoding phospholipases using (i) nucleotides 568 to 2045 of SEQ ID NO:1, (ii) the cDNA sequence contained in nucleotides 568 to 2045 of SEQ ID NO:1, or (iii) a complementary strand of (i) or (ii) (page 5, line 1, to page 7, line 7); for comparing the percent identity of the deduced amino acid sequences of the phospholipases to amino acids 20 to 464 of SEQ ID NO: 2 using the Clustal method according to Higgins, 1989, *CABIOS* 5: 151-153 (Example 4); for determining the degree of homology between two nucleic acid sequences using the Wilbur-Lipman method according to Wilbur and Lipman, 1983, *Proceedings of the National Academy of Science USA* 80: 726-730 (page 12, line 29, to page 13, line 8); for producing the phospholipases (Example 5); and for purifying the phospholipases and characterizing the properties of the encoded phospholipases (Examples 6-9). It is well within the skill of the art to isolate and identify the claimed nucleic acid sequences using the Applicants' disclosure.

In support of Applicant's arguments is the Declaration of Dr. Randy Berka. Dr. Berka conducted a BLASTP search of several publicly available protein databases (NR, PIRNREF, GENESEQP, SWALL) using SEQ ID NO: 2 as the query sequence to determine whether SEQ ID NO: 2 could be used to identify homologues that encode a phospholipase subsequent to the filing date of the instant application. The results of the search revealed four proteins having phospholipase activity from *Aspergillus niger*, *Oryza sativa*, and *Burkholderia pseudomallei*. Dr. Berka states: "These results clearly demonstrate the ability to use SEQ ID NO: 2 to identify proteins having phospholipase activity from other sources. A skilled person would be able to isolate the gene encoding these phospholipases using Applicants' specification."

With regard to man-made variants, Dr. Berka states: "[A] skilled person could easily prepare a variant of SEQ ID NO: 2 containing a deletion, insertion, and/or substitution of one or more amino acid residues. The specification on page 7, lines 8-30, provides that conservative amino acid substitutions can be made that do not significantly affect the folding and/or activity of SEQ ID NO: 2 and provide examples of conservative substitutions within the group of basic amino acids, acidic amino acids, polar amino acids, hydrophobic amino acids, aromatic amino acids, and small amino acids. Amino acid substitutions that do not generally alter the specific activity are described, for example, by H. Neurath and R.L. Hill, 1979, *In, The Proteins*, Academic Press, New York. The specification on page 13, line 9, to page 14, line 4, further describes methods for identifying amino acid residues essential to the activity of a phospholipase B. In fact, the findings of Guo *et al.* (2004) support the contention that proteins are generally tolerant to amino acid substitutions, and the probability of destroying protein function is surprisingly small."

The Office alleges that "it is <u>not</u> routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims ..." Dr. Berka disagrees with this statement and states: "As of October 1999, a skilled person was able to routinely produce thousands of mutants of SEQ ID NO: 1 through mutagenesis and other techniques and screen the mutants in a short period of time without undue experimentation. See, for example, Christians *et al.*, 1999, *Nature Biotechnology* 17: 259-264; Zocher *et al.*, 1999, *Analytica Chimica Acta* 391: 345-351; Rieger *et al.*, 1999, *Yeast* 15: 973-986; Genome Analysis, A Laboratory Manual, Volume 3, Cloning Systems, Robotic Replication pp. 20-22, Cold Spring Harbor Laboratory Press, 1997; Kell, 1999, *Trends in Biotechnology* 17: 89-91; and Dove, 1999, *Nature Biotechnology* 17: 859-863; Armstrong *et al.*, 1998, *Journal of Biomolecular Screening* 3: 271-275; Eickhoff *et al.*, 1999, *BioMethods* 10: 17-30; and Stevens *et al.*, 1998, *Journal of Biomolecular Screening* 3: 305-311. In addition, the specification provides on page 13, line 9, to page 14, line 4, how to identify essential amino acids in the sequence of SEQ ID NO: 2." One skilled in the art can, therefore, predict with reasonable statistical accuracy which modifications, if any, would result in a loss of the desired activity/utility..

For the foregoing reasons, Applicants submit that the claims overcome this rejection under 35 U.S.C. § 112. Applicants respectfully request reconsideration and withdrawal of the rejection.

III. Conclusion

In view of the above, it is respectfully submitted that all claims are in condition for allowance. Early action to that end is respectfully requested. The Examiner is hereby invited

to contact the undersigned by telephone if there are any questions concerning this amendment or application.

Respectfully submitted,

Date: November 23, 2004

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